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High-throughput epitope identification for snakebite antivenom

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Introduction

Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A2 in the venomus used for production of the investigated antivenom, this study focuses on these toxin families.

Objectives

- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

Epitopes locate to surface regions

To identify epitopes the observed peptide specific signal intensities were mapped back to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and Lys49-phospholipase A2 from Bothrops asper (venom used in antivenom production) are presented here.

Immunization mixture²

<table>
<thead>
<tr>
<th>Major venom components</th>
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<tr>
<td>Phospholipase A2</td>
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<tr>
<td>Metalloproteinase</td>
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</table>

Studying linear epitopes using peptide microarrays

Effect on cross-recognition

The n-hulls shaped red epitope in the B. asper metalloproteinase is found to be highly conserved among pit vipers metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight epitope residues and mean signal intensity of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, binding is still observed in all of the corresponding eight 15-mer peptides, although the microarray signals are reduced up to seven times (data not shown).

These results suggest that ICP Crotalidae polyvalent antivenom might offer protection from the investigated metalloproteinases, including the toxins from the Asian Gloydus species if these in vitro experiments translate to the in vivo situation.

Conclusions

- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

Acknowledgement

The peptide microarray experiments were performed at Schäfer-N. Copenhagen. We would like to thank Claus Schaar, Christian Akjed Hansen, and Jens Kerings for experimental setup and support. We further thank the Novo Nordisk foundation for financial support (grant number: NNF13OC0016131).

References


CLUSTAL O(1,2.1) multiple sequence alignment

Mean AU overlapping peptides

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<thead>
<tr>
<th>QUERY</th>
<th>Sequence</th>
<th>Similarity</th>
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</table>

The table above shows the mean AU overlapping peptides for the query sequence. The similarity is calculated based on the CLUSTAL O alignment method.

Data analysis and protein modeling

Antibody binding and detection

Synthesis on microarray

The figure above shows the synthesis on microarray using the CLUSTAL O alignment method. The antibody binding and detection are indicated by the red and blue colors, respectively.

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